Mushroom Discoloration: New Processes For Improving Shelf Life & Appearance

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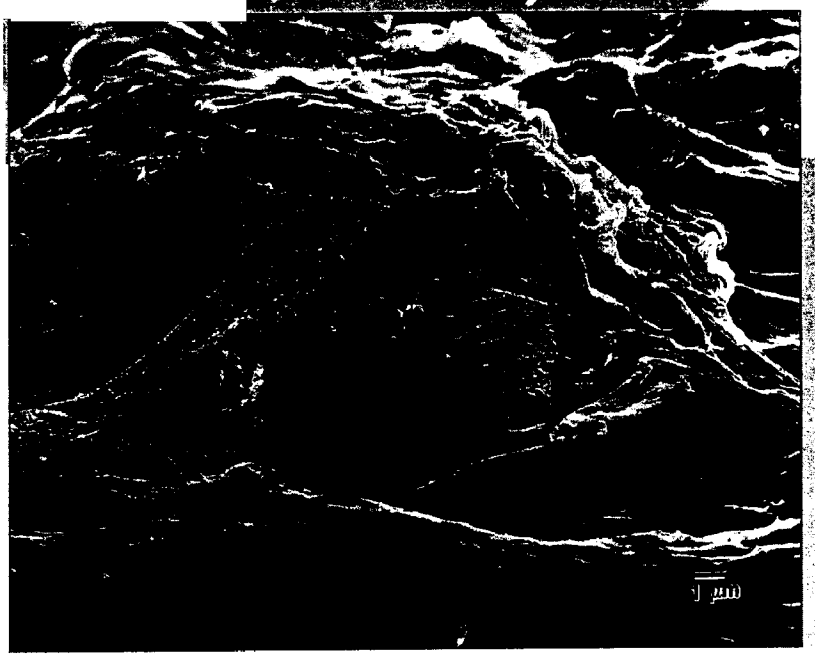

Figure 1. Scanning electron micrograph of hyphae in lesion on surface of washed mushroom, showing mechanical damage and presence of bacteria. Horizontal bar = 1 micrometer.

ABSTRACT

Quality and shelf-life defects associated with washing of mushrooms were investigated. Purple discolorations were attributed to the reaction of an intermediate in the tyrosinase-mediated oxidation of L-DOPA with sinapic acid. Sunken lesions were bacterial in origin. Both defects could be prevented by treatment of mushrooms with hydrogen peroxide vapor or solution. Enzymatic browning of mushroom surfaces could be inhibited by application of a dip containing sodium erythorbate and other GRAS browning inhibitors. Use of a 3-5% hydrogen peroxide dip followed by the browning inhibitor dip, as a two-stage wash, can control bacterial spoilage and enzymatic browning of mushrooms for about one week at 4°C.

INTRODUCTION

Our laboratory has been conducting research on the quality and shelf life of minimally processed fruits and vegetables. Much of our effort has been concerned with controlling enzymatic browning and finding effective replacements for sulfites which have been banned in most fresh products. Several years ago, we became involved in a research project concerning a unique discoloration seen in washed mushrooms. This study was carried out under a Cooperative Research and Development Agreement between the Agricultural Research Service and six Cooperating Companies, who partially funded the research (Sapers et al., 1993).

In this paper, the results of that study will be reviewed, and several new treatments that may significantly improve the shelf life and appearance of fresh mushrooms will be described.

RESEARCH ON WASHED MUSHROOMS

MICROSCOPY & MICROBIOLOGY

When fresh mushrooms are washed, adhering casing material is removed, thereby improving product appearance. The mushrooms can then be sliced for direct addition to salads and other foods. As an additional benefit, we found that washed mushrooms consistently showed less browning than unwashed mushrooms (indicated by a decrease in L*-value at the pileus surface during storage, measured by tristimulus colorimetry, and visual observation), provided that they did not undergo spoilage first (Table 1). Our studies suggest that this is due to leaching of browning precursors during washing (Choi and Sapers, 1994a). However, washed mushrooms are more perishable than unwashed mushrooms. During storage, purple or gray blotches appear on the cap surface of washed mushrooms after about 4-6 days. Gradually, these blotches evolve into sunken lesions.

Using scanning electron microscopy, we examined the surface structure of mushrooms showing blotches and lesions. The cap surface of normal unwashed mushrooms has an open structure of intertwined hyphae with few visible bacterial cells. Mechanical damage can be seen on the cap of washed mushrooms. The site of a lesion on the cap surface is matted, individual hyphae appear ragged and collapsed, and large numbers of rod-shaped bacteria are present (Fig. 1). We found that many of the bacteria were fluorescent pseudomonads, including the well-known mushroom pathogen Pseudomonas tolaasii which produces dark, sunken lesions on mushroom surfaces. Other isolates, identified as

Pseudomonas reactants, the species used to detect Pseudomonas tolaasii by the "white line" test, produced a brown lesion that was not sunken (Wells et al., 1994).

When mushrooms are washed, they absorb as much as 8% of their fresh weight, depending on the degree of agitation used. We found that the tendency of mushrooms to develop lesions was greater when the mushrooms absorbed more water. Lesion development could be suppressed by addition of an antibiotic such as streptomycin, which would not be permitted in foods, or by addition of acidic polyphosphates.

DISCOLORATION OF WASHED MUSHROOMS

Experiments in which a purple color was induced by application of L-DOPA, a phenolic amino acid found in mushrooms, to the surface of washed mushrooms, suggested that the purple blotch preceding lesion development was caused by some form of enzymatic browning. However, purpling could not be prevented by prior treatment of mushrooms with browning inhibitors such as sulfite or ascorbic acid.

Studies by Choi and Sapers (1994b) indicated that the purple discoloration probably resulted from a reaction involving a short-lived intermediate in the enzymatic browning of L-DOPA, a well known phenolic compound in mushrooms (Fig. 2). This intermediate may react with another phenolic compound found in mushrooms, sinapic acid, or its oxidation product, to form a stable, purple-colored reaction product. These reactions might be triggered by injury to hyphae during washing or by the metabolic activity of bacteria.

PROPOSED SOLUTION TO PROBLEM

From our understanding of the bacteriological and chemical basis of defects resulting from washing, we visualized a potential solution to this problem entailing two successive treatments. First, by treating mushrooms with a bactericide to reduce the numbers of

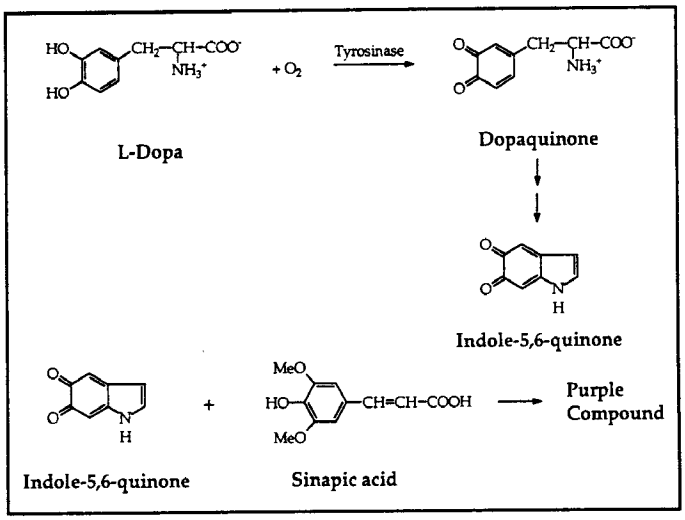

Figure 2. Reactions involved in formation of purple compound in washed mushrooms.

spoilage bacteria on mushroom surfaces, we should be able to prevent lesion development. Then, by applying a browning inhibitor wash, we could control discoloration reactions. Washing treatments should be sufficient to remove adhering soil but brief enough to avoid excessive water uptake which would favor subsequent spoilage.

HYDROGEN PEROXIDE VAPOR TREATMENTS

Forney *et al.* (1991) demonstrated that post harvest decay of table grapes could be inhibited by treatment with hydrogen peroxide vapor. In a study of sulfate alternatives at the Pennsylvania State University, McConnell found that the shelf life of mushrooms, washed with a solution containing 1% hydrogen peroxide and 0.1% calcium-disodium EDTA, was greater than that obtained with sulfite and similar to that of unwashed mushrooms (McConnell, 1991: Mau *et al.*, 1993). Our first approach was to use heated hydrogen peroxide vapor to kill spoilage bacteria on mushroom surfaces. We did not choose sodium hypochlorite as the bactericide because this compound induces browning at concentrations of 50 ppm or higher. We assembled a small-scale system for treating mushrooms with hydrogen peroxide vapor (Fig. 3) and then evaluated the system with mushrooms intentionally inoculated with Pseudomonas tolaasii at levels high enough to produce lesions during storage. This treatment was effective in delaying lesion formation in mushrooms inoculated with as many as 10 million (10^7) bacterial cells and in preventing lesion formation at lower inoculation levels (Table 2). A parallel experiment in which water was substituted for hydrogen peroxide demonstrated that our results were due to hydrogen peroxide exposure and not to the elevated temperature.

Hydrogen peroxide vapor exposure leaves no residues on treated mushrooms because mushrooms contain the enzyme catalase which rapidly breaks down residual peroxide to water and oxygen. Treated mushrooms show no off-flavors or textural abnormalities. However, L*-value measurements and visual observation indicated that the

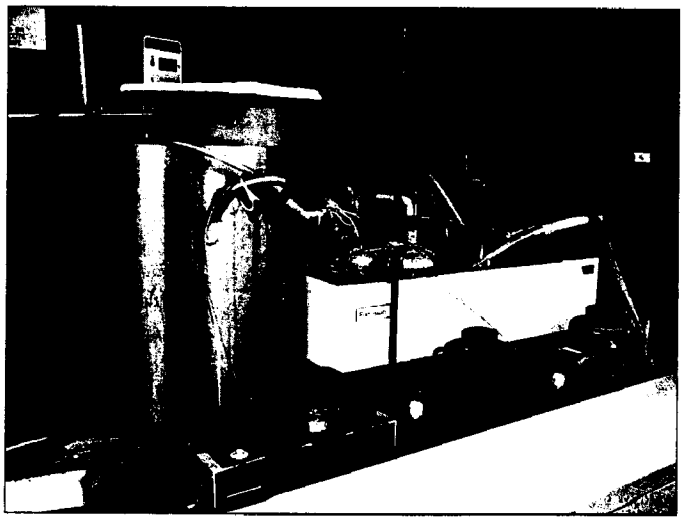

Figure 3. Laboratory-scale system for treating mushrooms with hydrogen peroxide vapor.

hydrogen peroxide vapor treatment induced browning in mushrooms during storage (Table 3). We found that browning could be delayed by immersing treated mushrooms in a browning inhibitor solution (see section on development of effective browning inhibitors). In recent experiments, we have been able to reduce browning further by using shorter exposures to hydrogen peroxide vapor, a lower treatment temperature, and a more effective browning inhibitor dip.

HYDROGEN PEROXIDE DIPS

As an alternative to use of hydrogen peroxide vapor, we found that dipping mushrooms in a hydrogen peroxide solution also was effective in extending shelf life but caused less browning than the vapor treatment. We use a 30-second immersion in 3-5% peroxide solution to avoid excessive water uptake. At the lower concentration, which is the same as that of the household hydrogen peroxide product, lesion formation in mushrooms inoculated with Pseudomonas tolaasii was suppressed. These hydrogen peroxide solutions are relatively stable and safe to handle. As with the vapor treatment, mushrooms dipped in a hydrogen peroxide solution show no sensory defects other than a tendency to brown. However, browning can be controlled for at least 6-8 days by application of a browning inhibitor dip after the peroxide dip. Thus, by use of this two-stage dipping treatment, we can wash mushrooms without accelerating spoilage and, at the same time, control enzymatic browning. We are hopeful that the two-stage treatment will continue to show promise and be economically viable when evaluated under actual production conditions.

TABLE 1

Effect of Commercial Washing Treatment On Browning of Mushrooms

	L*[a] Day			Appearance on Day 4	
				Browning[f]	Lesion[g]
Sample	0	2	4	(%)	%
First break, not washed	86.6[d]	84.3[e]	83.6[e]	96	10
First break, washed	88.6[c]	88.1[c]	87.8[c]	44	18
Second break, not washed	87.9[c]	86.9[d]	85.7[d]	86	10
Second break, washed	89.8[b]	89.6[b]	89.0[b]	0	22

[a] Reflectance measured at top of pileus.
[b-e] Means of 50 replicates; means within columns, followed by different superscripts, are significantly different at $p<0.05$ by the Bonferroni LSD test.
[f] Percent of sample (50 mushrooms) with moderate or severe browning.
[g] Percent of sample (50 mushrooms) with bacterial lesions.

TABLE 2

Effect of H_2O_2 Vapor Treatment on Mushrooms Inoculated with *Pseudomonas tolaasii*.

		Lesion Formation[b,c] Inoculum (cells/mushroom)		
Treatment[a]	Exposure time (min)	10^6	3×10^6	10^7
Control	0	+	++	++
H_2O_2	30	-	±	++
	45	-	-	+
	60	-	-	+
H_2O	30	+	+	++
	45	+	+	++
	60	+	++	++

[a] 6 L/min at 43°C equivalent to 10 µg $H_2O_2/cm^2/min$.
[b] Incubated at 4°C for 7-9 days.
[c] Large ++, Small +, Marginal ±, None -.

TABLE 3

Occurrence of browning in mushrooms treated with hydrogen peroxide vapor[a]

	L*[b] Day			Browning[c] Day	
Hydrogen peroxide exposure time (min)	0	3	7	3	7
0	93.8	92.2	84.0	+	++
30	92.8	89.9	81.3	+/++	++
60	93.2	83.2	73.7	++/+++	+++

[a] Flow rate = 6 L/min; H_2O_2 temperature = 40°C; treatment chamber temperature = 50°C.
[b] Measured at top of pileus.
[c] +++ severe; ++ moderate, + slight.

CONTROL OF BROWNING IN MUSHROOMS

DEVELOPMENT OF EFFECTIVE BROWNING INHIBITORS

As part of our ongoing research on the development of sulfite replacements for minimally processed fruits and vegetables, we screened a number of browning inhibitors for whole and sliced mushrooms (Sapers et al., 1994). Inhibitors were applied individually or in combinations, in some cases with antimicrobial compounds, and under different conditions of pH, concentration and dipping time (Table 4). Many of these inhibitors were ineffective with mushrooms and individual compounds generally were less effective than combinations of several browning inhibitors.

One of our most effective browning inhibitor formulations is a combination o

4.5% sodium erythorbate, 0.2-0.4% cysteine hydrochloride, and 1000 ppm EDTA (disodium calcium salt), adjusted to pH 5.5 with sodium hydroxide. Sodium erythorbate has been used as a browning inhibitor for mushrooms previously. The other ingredients also are classified as GRAS (Generally Recognized As Safe) for specific food applications. FDA approval probably would be required before this combination could be used on mushrooms. The effectiveness of this formulation in controlling browning of external and cut surfaces of mushrooms can be seen in photographs of treated and control mushrooms after 1 week of storage at 4°C (Fig. 4).

Application of this browning inhibitor treatment to mushrooms dipped for 30 sec in 5% hydrogen peroxide was highly effective in controlling browning during storage (Table 5). The peroxide treatment prevented development of lesions that were seen when the browning inhibitor dip was used alone.

TABLE 4

Treatments to Control Defects of Minimally Processed Mushrooms.

Browning Inhibitors	Antimicrobials
Ascorbic acid	Streptomycin sulfate
Sodium ascorbate	Hydrogen peroxide
Erythorbic acid	Potassium sorbate
Sodium erythorbate	Sodium benzoate
Ascorbic acid-2-phosphate	Sodium hypochlorite
Citric acid	
Sodium acid pyrophosphate	**Other Variables**
EDTA	pH
4-Hexylresorcinol	Concentration
Cysteine	Combinations
Zinc chloride	Dipping time
Hydroxypropyl ß-cyclodextrin	

RELATIONSHIP OF BROWNING TO ENVIRONMENTAL FACTORS

In the course of our mushroom trials, we have observed that first flush mushrooms consistently show more browning than second flush mushrooms (see Table 1). Also, we found that mushrooms grown on a largely synthetic casing layer showed less browning during storage than mushrooms grown on a casing layer prepared from recycled compost. These observations suggest that environmental conditions during mushroom production can have a large effect on the tendency of mushrooms to brown. Further research in this area might lead to the identification and control of specific environmental factors affecting browning of mushrooms.

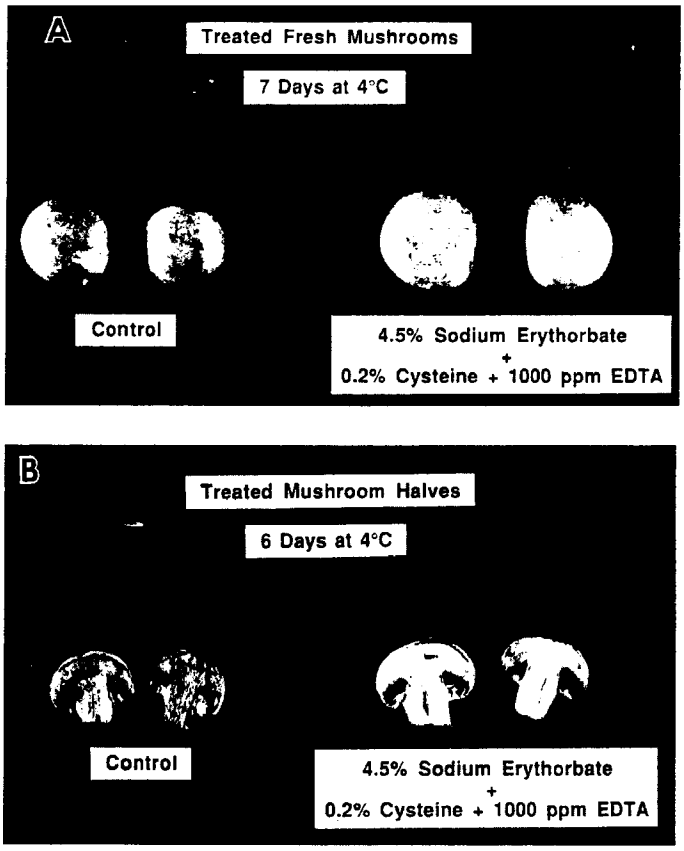

Figure 4. Response of mushrooms to treatment with browning inhibitor dip: A.Whole mushrooms; B. Cut surface of mushroom halves.

FUTURE RESEARCH

We have recently initiated a new cooperative research project to commercialize the two-stage, hydrogen peroxide-browning inhibitor dipping process. This project will focus on treatment optimization, scale-up, field testing under commercial conditions, and cost analysis.

We also plan to continue our research on optimization of hydrogen peroxide vapor treatments and on the development of more efficient methods of washing mushrooms. We hope that the technology developed in these studies will result in improvements in mushroom quality and shelf life.

REFERENCES

CHOI, S.-W. AND SAPERS, G. M. (1994a). Effect of washing on polyphenols and polyphenol oxidase in commercial mushrooms (Agaricus bisporus). J. Agric. Food Chem. 42: 2286-2290

CHOI, S.-W. AND SAPERS, G. M. (1994b). Purpling reaction of sinapic acid model systems containing L-DOPA and mushroom tyrosinase. J. Agr. Food Chem. 42: 1183-1189.

FORNEY, C. F., RU, R. E., DENIS-ARRUE, and SMILANICK, J. L. (1991).

Vapor phase hydrogen peroxide inhibits postharvest decay of table grapes. HortScience 26: 1512-1514

MAU, J. L., MIKLUS, M. B., and BEELMAN, R. B. (1993 The

shelf life of Agaricus mushrooms. In Shelf Life Studies of Foods and Beverages, G. Charalambous (Ed.), Elsevier Science Publishers.

McCONNELL, A. L. 1991. Evaluation of wash treatments for the improvement of quality and shelf-life of fresh mushroom (Agaricus bisporus). M.S. thesis. Department of Food Science, The Pennsylvania State University, University Park, PA.

SAPERS, G. M., CHOI, S.-W., MILLER, R. L. AND WELLS, J. M. (1993). USDA mushroom quality research project shows progress. Mushroom News 41 (9): 4-5.

SAPERS, G. M., MILLER, R. L., MILLER, F. C., COOKE, P. H. AND CHOI, S.-W. (1994). Enzymatic browning control in minimally processed mushrooms. J. Food Science. 59: 1042-1047

WELLS, J. M., SAPERS, G. M., FETT, W. F., BUTTERFIELD, J. E., JONES, J. B., BOUZAR, H. AND MILLER, F. C. (1994). Postharvest discoloration of the cultivated mushroom Agaricus bisporus in Pennsylvania caused by Pseudomonas tolaasii, P. 'reactants' and P. 'gingeri'. Submitted to Phytopathology.

TABLE 5

Effect of Hydrogen Peroxide and Browning Inhibitor Dips on Appearance of Whole Mushrooms

	Stored at 4°C L[b]			Browning[c]	
	Day			Day	
Treatment[a]	0	4	7	4	7
Control	93.6	90.0	88.2	+/+++	++/+++
Browning Inhibitor Only	93.1	88.3	84.2	+/++(L)	++/+++(L)
Hydrogen Peroxide + Browning Inhibitor	92.1	91.1	89.1	+	+

[a] 30 sec in 5% hydrogen peroxide, followed by 20 sec in 4.5% sodium erythorbate + 0.4% cysteine + 1000 ppm EDTA, pH 5.5.
[b] Measured with spectrocolorimeter at top of pileus.
[c] +++ severe; ++ moderate; + slight.
L = lesions.

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